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Clinicopathological study on two types of cryptogenic organizing pneumonitis

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Clinical and pathological studies on cryptogenic organizing pneumonitis (COP) were performed in 19 cases diagnosed with transbronchial lung biopsy (TBLB). All patients suffered from fever and several respiratory symptoms. Laboratory data showed increases in erythrocyte sedimentation rate, positivity for C-reactive protein, negative tuberculin reactions and increases in complement level. Pathological findings demonstrated that there were two kinds of organizing processes. Fourteen of the 19 cases were treated with prednisolone, and two cases were observed without administration. The remaining three cases could not be followed up after therapy. In 11 of the 16 cases, abnormal shadows in chest X-ray disappeared, but remained present in five cases. As for the relationship between pathological findings and shadows in chest X-ray, Masson bodies without fibrin were observed in the 11 cases which were without shadows on X-ray, but Masson bodies containing or related to fibrin were observed in the five cases in which abnormal shadows remained.

These results suggest that there are two types of organizing process in COP. Type I is an unexplained organizing process in which fibrin is not present or involved. It responds well to steroids and the prognosis is favourable. Type II is an organizing process which involves fibrin, and the character of the fibroblast-like cells is very similar to that of myofibroblasts. Type II organizing process responds poorly to steroids. Both processes can be notified relatively easily, even by TBLB tissues.

Introduction

Recently, many patients with pneumonia show poor resolution of chest shadows and require differentiation from other diseases. In such patients, transbronchial lung biopsy (TBLB) often reveals organization in the alveoli. The aetiology is generally unknown. This has been designated as cryptogenic organizing pneumonitis (COP) (1) and bronchiolitis obliterans organizing pneumonia (BOOP) (2). To explain this clinical phenomenon by comparison with pathologic findings, 19 cases which were diagnosed as COP by TBLB were evaluated clinically and pathologically.

Materials and Methods

The 19 subjects were patients who had been admitted with pulmonary involvement of the pneumonia type, but did not respond to antibiotics and were shown to have organizing pneumonia (OP) histologically by TBLB between January 1989 and December 1992. In these patients, no response was observed to various antibiotics and the cause of the disease was serologically and clinicopathologically unknown. The characteristics of these patients, signs and symptoms, results of clinical laboratory tests, pathologic findings of TBLB specimens and therapeutic results were evaluated. The material obtained by TBLB was fixed with buffered formalin and stained with HE. Additional phosphotungstic acid–haematoxylin (PTAH), Azan, Elastic van Gieson (EVG), and Alcian blue staining were made depending on the case. Also, to clarify the properties of cells proliferating in Masson bodies, deparaffinized sections were studied using immunostaining by the avidin–biotin complex (ABC) method with peroxidase. Anti-macrophage, anti-muscle-actin (m-actin) (Enzo Diagnostics Inc., U.S.A.), anti-epithelial membrane antigen (EMA), anti-vimentin, anti-desmin (Dakopatts, Denmark), and anti-α-smooth muscle-actin (α-sm-actin) (BioMaker, Israel) antibodies were used. Histological findings were examined in Masson bodies, alveoli and interalveolar septa. Fibrin in Masson bodies, fibroblasts, alveolar macrophages, type II alveolar epithelium, oedema of interalveolar septa, fibrosis, and inflammatory cell infiltration were graded as $-$, $+$, and $++$, and their correspondence with clinical findings was evaluated.

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Table 1 Clinical features

Case No.	Age (yr)	Sex	Fever	Cough	Sputum	Dyspnoea	PO_2 (mmHg)	PCO_2 (mmHg)	Therapy (PSL mg)	Chest X-ray shadows after therapy
1	70	M	+	+	+	+	79·4	42·6	Pulse	1
2	86	M	+	−	−	+	53·7	36·3	60	D
3	89	M	+	−	−	+	61·4	40·1	60	1
4	95	M	+	+	+	+	48·9	28·6	Pulse	1
5	61	M	−	−	−	+	75·9	35·9	20	D
6	62	F	+	+	+	+	71·4	34·4	60	D
7	58	M	+	+	+	−	76·9	45·8	30	I
8	73	M	+	+	+	−	74·0	34·5	60	D
9	88	M	+	+	+	−				
10	75	M	−	−	−	−				
11	67	M	+	+	−	−			15	D
12	64	F	+	+	−	−			20	D
13	34	F	−	+	+	−			0	D
14	63	M	+	+	+	−	71·3	36·3	0	D
15	72	M	+	+	+	+	55·8	42·8	Pulse	I
16	77	F	+	+	+	+	56·5	39·2	60	D
17	70	M	+	+	+	−			Pulse	D
18	62	M	+	+	+	−	74·7	38·5		
19	54	F	+	+	+	−			20	D

+, present; −, absent; D, disappeared; I, improved but not disappeared.

Table 2 Laboratory data*

Case No.	WBC	ESR (mm hr^{-1})	CRP (mg dl^{-1})	LDH (IU l^{-1})	ChE (ΔpH)	TP (g dl^{-1})	CH50 (ml^{-1})	RA (IU ml^{-1})	ANF	PPD test	IgG (mg dl^{-1})
1	5500	115	0	218	0·69	7·8	64·0		−	−	985
2	7900	76	6·0	245	0·38	5·7	46·6	−	−	−	2606
3	6600	75	9·6	295	0·39	5·9	31·6	−	−	−	864
4	7100	38	13·5	353	0·53	4·8	39·8	34	−	−	1270
5	5600	91	17·9	369	1·03	6·3	59·9	−	−	−	1320
6	9600	102	3·0	243	0·50	6·4	65·0	−	−		1372
7	15 900	88	10·1	245	0·38	5·8	44·9		−	+	2380
8	6100	115	11·2	293	0·64	7·3	65·0	−	−	+	1700
9	5800	18	2·0	323	0·35	6·3	37·0	−	−	−	1570
10	3000	42	4·0	396	0·52	6·1					
11	5100	78	15·1	237	0·40	6·1		−	−		
12	8900	54	9·9	322	0·61	6·2	45·7	−	−	−	1690
13	5600	96	2·9	210	0·97	7·4	32·5	−	−	−	2043
14	9300	98	5·9	324	0·29	7·1	37·8	31	−	+	2575
15	9900	129	26·1	382	0·45	5·7		−	−	−	
16	7400	95	5·1	303	0·63	6·8	44·9	−	−	+	1845
17	6600	72	4·2	363	0·58	7·6		−	−	−	
18	10 600	130	6·9	353	0·63	6·6	31·7	−	−	−	
19	13 300	112	13·6	322	1·14	8·3	55·4	−	−	−	2128

*Abbreviations see text.
+, positive result; −, negative result.

Results

As shown in Table 1, the patients were aged 39–95 years with a mean of 69·5 years. They consisted of 14 males and five females. Concerning the primary complaints, some respiratory symptoms were observed in all patients, and fever was noted in 16 of the 19 patients. Dyspnoea was present in eight patients. Table 2 summarizes the results of laboratory tests on

Table 3 Pathological findings

Case No.	Masson bodies				Alveoli			Septa		
					Type II cells					
	Fibrin	Spindle cells	Lining alveolar cells	Macrophages	Proliferation	Swelling	Macrophages	Oedema	Fibrosis	Inflammatory cells
1	++	+	−	+	+	+	+	−	+	+
2	−	++	+	+	++	+	−	−	+	−
3	++	++	−	+	−	−	+	+	+	−
4	+	++	+	+	+	+	+	+	+	+
5	−	++	+	+	+	+	++	−	+	−
6	−	+	+	+	+	−	+	−	++	+
7	+	++	+	+	+	−	+	+	+	+
8	−	++	+	+	++	−	+	+	+	+
9	−	++	+	−	−	+	++	+	++	+
10	−	+	−	−	−	−	−	−	−	−
11	−	++	+	−	+	++	+	−	−	−
12	−	+	−	−	+	−	+	−	+	+
13	−	++	+	−	+	−	+	+	+	+
14	−	+	−	+	++	+	+	+	+	+
15	++	+	+	++	+	+	+	+	++	++
16	−	+	+	++	+	+	+	−	+	+
17	−	+	−	+	−	−	+	+	−	+
18	−	+	−	+	−	−	+	+	−	+
19	−	+	+	+	+	−	+	+	−	+

+, ++, present to lesser, greater extent; −, absent.

admission. The white blood cell count (WBC) ranged from 3000–15 900 with a mean of 7884. Leucocytosis was only noted in five patients. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), on the other hand, were increased in all patients but one. Concerning biochemical examinations, GOT, GPT, and LDH were within the normal ranges, but cholinesterase (ChE) was reduced and the serum total protein (TP) level was reduced in many patients, suggesting nutritional inadequacy. Immunological examinations showed increases in whole haemolytic complement (CH50) level in seven of the 15 patients. Autoantibodies were negative except for two cases, who were positive for rheumatoid factor (RA test). The PPD test was performed in 15 patients, and 11 proved negative, suggesting decreases in cell-mediated immunity. However, immunoglobulin or γ-globulin was not reduced in any patient.

Concerning histopathologic findings, the patients could be divided into two groups according to the nature of Masson bodies, most importantly due to the presence or absence of fibrin in these bodies (Table 3). First, in the five fibrin-positive cases, the degree of organization of Masson bodies varied widely from slight to advanced fibrosis, showing proliferation of fibroblast-like cells and increase in collagen fibres [Plate 1(a)], accompanied by fibrotic thickening in the surrounding alveolar walls. Cells in Masson bodies included mainly macrophages, lymphocytes and plasma cells, in addition to bipolar and long spindle-shaped fibroblast-like cells. Some Masson bodies were covered by type II epithelium, connecting with other Masson bodies in adjacent alveoli through Kohn's pores. Fibrin in Masson bodies was stained by PTAH in the early stage, but later on PTAH stained positive in the cytoplasm of long spindle-shaped cells arranged concentrically in Masson bodies [Plate 1(b)]. It is of interest that anti-m-actin and anti-α-sm-actin antibodies were positive in the cytoplasm of these cells [Plate 1(c)], so that the cells are considered to have properties of myofibroblasts. In Masson bodies showing advanced organization, mature collagen fibres stained blue with Azan were increased, but no elastic fibres were involved in Masson bodies, as revealed by EVG staining. In 14 fibrin-negative cases, multipolar proliferative cells in Masson bodies were sparsely distributed and were rich in myxoid matrix [Plate 2(a)]. Surrounding interalveolar septa were similarly thickened. By AB staining, the matrix at Masson bodies was intensely stained [Plate 2(b)]. Anti-EMA antibody staining was linearly positive in the portions of the surface of the surrounding alveoli but not that of Masson bodies [Plate 2(c)]. In addition, these cells

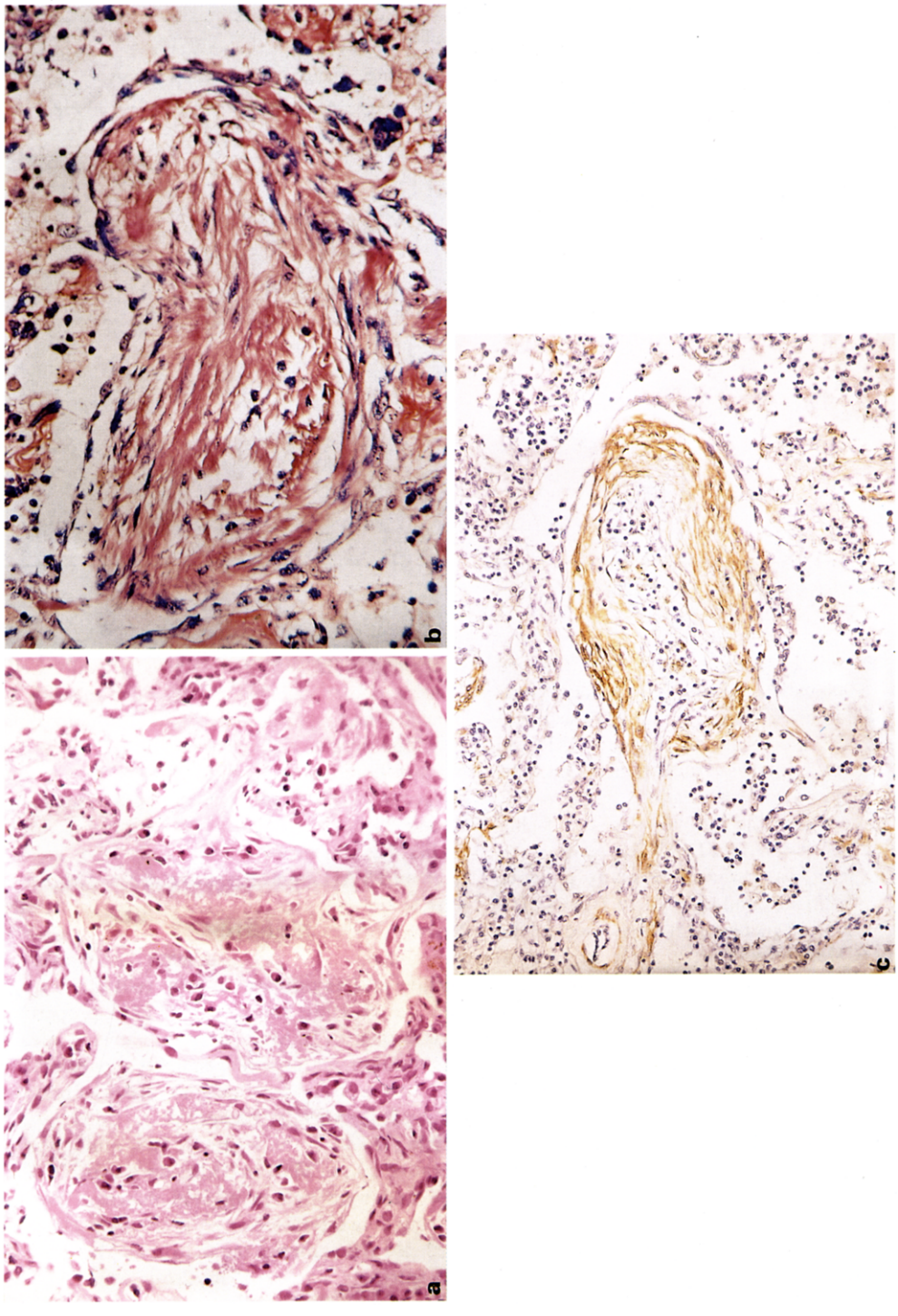

Plate 1 (a) Masson bodies at a relatively early stage in type II COP, containing fibrin. HE × 171, (b) Concentrically arranged spindle cells in a Masson body, revealing positive stainability with PTAH. × 171 . (c) Strong positive reaction of spindle cells with anti-m-actin antibody. ABC method × 108.

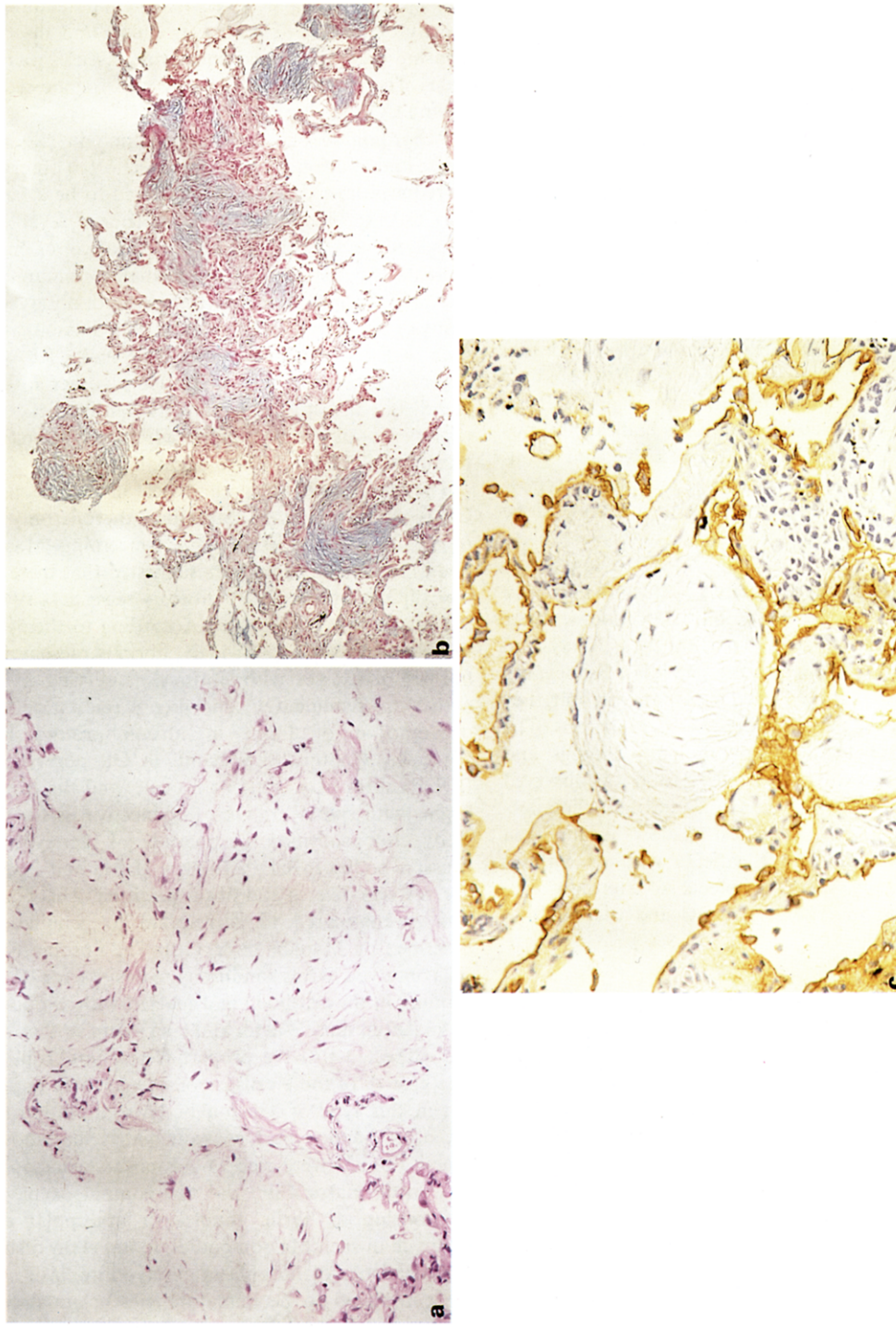

Plate 2 (a) Masson bodies observed in type I COP, showing loose cellularity and myxoid appearance. HE × 108. (b) Masson bodies positively stained with alcian blue. × 54. (c) Negative immunoreactivity of Masson body with anti-EMA antibody. ABC method × 171.

Table 4 Interrelationship between effects of steroid therapy and types of Masson bodies

		Type I	Type II
Shadows in chest X-ray after steroid therapy	Disappeared	11/11	0/5
	Improved but not disappeared	0/11	5/5

were negative for antibodies to m-actin or α-sm-actin. As shown in Table 3, findings in the two groups were similar except for the presence or absence of fibrin, and there were no particular changes. However, inflammation varied widely according to the degree of organization in fibrin-positive patients, possibly because of the time difference of sampling. Therefore, we pathologically classify Masson bodies into two types. The patients with fibrin-negative and α-sm-actin negative Masson bodies were defined as type I, and those with fibrin positive or α-sm-actin positive Masson bodies as type II.

Steroids were administered to 14 of the 16 patients who could be followed after TBLB, and two were observed without treatment. The chest shadows after treatment were followed up with chest X-ray and high resolution CT-scan. The shadows disappeared within 2 months in 11 of the 16 patients, but they persisted in five patients despite steroid therapy. The relationship between histopathologic findings and treatment was evaluated. A correlation was observed between the types of Masson bodies and the effectiveness of steroids. The chest shadows disappeared completely in all type I patients with or without steroid therapy, but the effects of steroids were not sufficient in type II patients and the shadows persisted (Table 4).

Discussion

Organizing pneumonitis is a pathologic diagnosis made on the basis of histological findings. Clinically, it refers to a condition in which fibrin-containing effusion in the alveolar cavity is organized due to disturbances of the healing process of bronchopneumonia or lobar pneumonia. The disease is generally called persistent pneumonia (3,4), but hypersensitivity pneumonitis and inflammation or neoplasm are often accompanied by OP as a partial phenomenon around the lesions. In our patients, some acute clinical symptoms and fever were observed, infiltration or mixed shadows were observed in chest radiograms, and bacteriological examination was negative. The patients were sero-negative for chlamydia and mycoplasma, there was no occupational history, history of keeping pets, environmental factors, or history of the use of particular drugs, and the relationship between OP and underlying diseases was not clear. Therefore, these patients were diagnosed as having COP (1).

According to our data, a reduction in cholinesterase and poor nutritional state were often observed, so reduced host defence is considered to be a factor in COP. In addition, the complement level was increased and PPD tests were negative in many patients, suggesting that cell-mediated immunity is impaired. Peripheral leucocytosis was not observed in many of our patients, but signs of acute inflammation such as fever and increases in ESR and CRP were observed in all patients, as reported by other authors (1,2,5–9). Investigation for autoantibodies was negative in all patients, unlike idiopathic interstitial pneumonia. LDH was also normal.

The literature about pathological investigations of OP is scarce (1,2,6). In particular, there is only the report of Manabe *et al.* (10) concerning Masson bodies in OP. These authors suggested that there are two different processes of fibrosis regardless of the site of the focus of fibrosis. According to them, the degree of fibrosis varies widely, fibrin is present, and fibrosis progresses with fibrin clots serving as the matrix in genuine OP, showing precipitation and organization of fibrin in alveoli around lung abscesses. On the other hand, in OP observed in BOOP, fibrin plays little or no role, and the disease begins with sparse growth of connective tissue. Our study also confirmed the presence of two types of Masson bodies in COP. In type II Masson bodies, fibrosis was diverse and fibrin persisted. This condition is considered to correspond to genuine OP described by Manabe *et al.* (10). The fibroblast-like cells observed in this condition are positive for PTAH staining in the cell body and reacted with antibodies to vimentin, muscle-actin and α-sm-actin so that they are suspected to have properties of myofibroblasts. The growth of these cells is considered to be important in this type of Masson body organization. On the other hand, type I Masson bodies that showed sparse fibrosis were rich in AB-positive mucopolysaccharide matrix, and did not contain or have a relationship to fibrin. Bipolar or multipolar cells observed in such Masson bodies do not show distinct characteristics of fibroblasts or myofibroblasts, and are considered to be immature mesenchymal cells. What causes the differences in these two types of Masson bodies? The period from the onset of symptoms to the diagnosis was $6 \pm 4{\cdot}1$ days (mean ± SD) in

type I patients and 12·8 ± 12·5 days (mean ± SD) in type II patients, which is not significantly different. Therefore, the differences in these two types of Masson bodies cannot be explained by the duration of illness. Katzenstein *et al.* (5) speculated that BOOP is caused by infection, especially viral. In our study, signs of inflammation were more marked in type II patients than in type I. The differences in these two types may only be caused by the magnitude of the insult. However, Masson bodies in type I closely resembled those occasionally observed in eosinophilic pneumonia and hypersensitivity pneumonitis, and Masson bodies in type II closely resembled those in pneumonia. Therefore, we believe that type I COP is caused by allergy and that type II COP by infection.

There are various reports on treatments for COP (1,8,11). Davison *et al.* (1) reported that COP responded well to steroids, but steroids are ineffective or worsen the condition in some patients. King *et al.* (11) reported the treatment outcome and prognosis on 96 subjects with COP, with complete remission in 63% of patients and partial remission in 31% of patients. In our study, steroids were effective in all patients. In particular, shadows disappeared from chest radiograms in all patients with type I Masson bodies after steroid therapy or without treatment. However, the shadows invariably persisted in all patients with type II Masson bodies despite steroid therapy, though they were reduced in size in some patients. These findings suggest a close relationship between the process of formation of Masson bodies in COP and the effects of steroids. This relationship has not been reported previously.

Recently, attempts at clinical classification of BOOP have been reported. Cordier *et al.* (7) divided patients with BOOP into those who showed multiple patchy shadows in chest X-rays (group 1), those who showed solitary shadows (group 2), and those with interstitial shadows (group 3). This study found that the outcome was favourable despite severe signs of inflammation such as cough, fever, and increased erythrocyte sedimentation rate in group 1. However, dyspnoea was severe and the outcome poor in some patients of group 3. We would suggest the pathological classification of COP and BOOP into two types according to the presence or absence of fibrin and to a positive or negative reaction of α-sm-actin antibody in Masson bodies, rather than by chest X-ray findings. The group with fibrin-negative, α-sm-actin negative Masson bodies is defined as type I, and the group with fibrin-positive or α-sm-actin positive Masson bodies as type II. Differences in clinical symptoms and chest X-ray findings between the two groups must still be clarified. However, this classification allows distinction of two groups of patients different in pathologic findings, responses to steroids and prognosis, and suggests two different disease types. Since epithelial injury is similar in BOOP, acute interstitial pneumonia (AIP) and usual interstitial pneumonia (UIP), Myers and Katzenstein (12) considered that both diseases are caused by acute epithelial injury. Fibroblasts and myofibroblasts are considered to migrate into alveoli because of this epithelial injury and deposit organizing effusions (13,14). According to Myers and Katzenstein, while this epithelial injury is localized around the airway in BOOP, it extends widely to the lung periphery in UIP and AIP, hence differences in clinical symptoms and prognosis (12). In contrast, we suspect that differences in the process of organization according to the presence or absence of fibrin in Masson bodies are responsible for differences in clinical symptoms and prognosis.

From these observations, we conclude that there are two types of organization in COP and BOOP. Type I is unexplained fibrosis, characterized by an abundance of myxiod matrix in which fibrin is not present or involved. It responds well to steroids, shadows in chest radiograms are shortly resolved and the prognosis is favourable. Type II is fibrosis which involves fibrin and the character of the fibroblast-like cells is very similar to that of myofibroblasts. It responds poorly to steroids and resolution of shadows in chest radiograms is delayed. The fact that the outcome of COP and BOOP can be estimated by histopathological examination of TBLB specimens is considered to be good news for clinicians.

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